SUMMARY

In one aspect, the present disclosure refers to a binding agent comprising an antigen-binding site that specifically binds an HLA PHOX2$_{BQYNPIRTTF}$ complex wherein: the HLA PHOX2$_{BQYNPIRTTF}$ complex comprises a peptide having the sequence QYNPIRTTF (SEQ ID NO: 1), an HLA α-chain polypeptide and a β_2 microglobulin polypeptide; the binding agent binds the HLA PHOX2$_{BQYNPIRTTF}$ complex presented by HLA-A*24:02, HLA-A*23:01, and HLA-B*14:02; and the binding agent is an antibody or antibody fragment. In another aspect, the present disclosure refers to a chimeric antigen receptor (CAR), a Killer Ig-Like receptor (KIR), or a Bispecific T cell-like Engager (BiTE) comprising the binding agent as described herein. In yet another aspect, the present disclosure refers to an isolated polynucleotide comprising a nucleic acid sequence encoding the binding agent, the CAR, the KIR, or the BiTE as described herein. In a further aspect, the present disclosure refers to an expression vector comprising the polynucleotide described herein operatively linked to a cis-acting regulatory element. In another aspect, the present disclosure refers to a cell comprising the polynucleotide or the expression vector as described herein. In one aspect, the present disclosure refers to a pharmaceutical composition comprising the binding agent, the CAR, the KIR, the BiTE, the isolated polynucleotide, the expression vector or the cell as described herein. In another aspect, the present disclosure refers to a method of detecting a cancer cell, comprising contacting the cell with the binding agent described herein, under conditions which allow the binding agent to bind to the HLA PHOX2$_{BQYNPIRTTF}$ complex, wherein binding of the binding agent to the HLA PHOX2$_{BQYNPIRTTF}$ complex or the level thereof is indicative of the cancer cell. In yet another aspect, the present disclosure refers to a method of identifying cancer in a subject in need thereof, comprising: a) detecting the presence of cancer cells in the subject according to the method described herein; b) identifying the subject as having cancer when cancer cells are detected. In a further aspect, the present disclosure refers to the use of the binding agent, the CAR, the KIR, the BiTE, the isolated polynucleotide, the vector, the cell, or the pharmaceutical composition described herein, in the manufacture of a medicament for treating cancer. In another aspect, the present disclosure refers to a CAR T cell comprising the CAR described herein.

The present disclosure describes a binding agent comprising an antigen-binding site that specifically binds an HLA PHOX2B$_{QYNPIRTTF}$ complex, comprising a peptide having the sequence QYNPIRTTF (SEQ ID NO: 1), an HLA α-chain polypeptide and a β_2 microglobulin polypeptide. The antigen-binding site may bind to the HLA PHOX2B$_{QYNPIRTTF}$ complex with

a dissociation constant (K_D) equal to or less than about 500 nM, equal to or less than about 200 nM, or equal to or less than about 13 nM. The binding agent may not MHC-restricted. The antigen-binding site may bind the HLA PHOX2B$_{QYNPIRTTF}$ complex presented by two or more of, three or more of, or four or more of HLA-A*24:02, HLA-A*23:01, HLA-B*14:02, HLA-C*07:01, HLA-C*06:02, HLA-A*29:02, and HLA-A*32:01.

The antigen-binding site may comprise:

a) a V_L comprising a CDR-L1 region as set forth in SEQ ID NO: 6, a CDR-L2 region as set forth in SEQ ID NO: 7, and a CDR-L3 region as set forth in SEQ ID NO: 8; and/or

b) a V_H comprising a CDR-H1 region as set forth in SEQ ID NO: 9, a CDR-H2 region as set forth in SEQ ID NO: 10, and a CDR-H3 region as set forth in SEQ ID NO: 11;

or

a) a V_L comprising a CDR-L1 region as set forth in SEQ ID NO: 15, a CDR-L2 region as set forth in SEQ ID NO: 16, and a CDR-L3 region as set forth in SEQ ID NO: 17; and/or

b) a V_H comprising a CDR-H1 region as set forth in SEQ ID NO: 18, a CDR-H2 region as set forth in SEQ ID NO: 19, and a CDR-H3 region as set forth in SEQ ID NO: 20;

or

a) a V_L comprising a CDR-L1 region as set forth in SEQ ID NO: 26, a CDR-L2 region as set forth in SEQ ID NO: 27, and a CDR-L3 region as set forth in SEQ ID NO: 28; and/or

b) a V_H comprising a CDR-H1 region as set forth in SEQ ID NO: 29, a CDR-H2 region as set forth in SEQ ID NO: 30, and a CDR-H3 region as set forth in SEQ ID NO: 31;

or

intracellular signaling domain may be CD3 epsilon, CD3 gamma, CD3 delta, TCR alpha, TCR beta.

Also described herein is an isolated polynucleotide comprising a nucleic acid sequence encoding the binding agent as described herein, or an expression vector comprising such as polynucleotide operatively linked to a cis-acting regulatory element. Also described is a cell comprising the such a polynucleotide or expression vector. Also provided is a pharmaceutical composition comprising the binding agent, the isolated polynucleotide, the expression vector or the cell.

In another example, there is described a method of detecting a cancer cell, comprising contacting the cell with the binding agent as described herein, under conditions which allow the binding agent to bind to the HLA PHOX2B$_{QYNPIRTTF}$ complex, wherein binding of the binding agent to the HLA PHOX2B$_{QYNPIRTTF}$ complex or the level thereof is indicative of the cancer cell. In yet another example, there is described a method of diagnosing and treating cancer in a subject in need thereof, comprising a) detecting the presence of cancer cells in the subject according to such a method, diagnosing the subject as having cancer when cancer cells are detected; and optionally treating the subject with an anti-cancer therapy. The cancer cell may be a neuroblastoma.

A further example describes for a method of treating a cancer, comprising administering to a subject in need thereof a therapeutically effective amount of the binding agent, the isolated polynucleotide, the vector, the cell, or the pharmaceutical composition as described herein. The cancer may be a neuroblastoma.

The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.” The word “about” means plus or minus 5% of the stated number.

It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein. Other objects, features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the disclosure, are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

WHAT IS CLAIMED:

1. A binding agent comprising an antigen-binding site that specifically binds an HLA PHOX2B$_{QYNPIRTTF}$ complex wherein: the HLA PHOX2$_{BQYNPIRTTF}$ complex comprises a peptide having the sequence QYNPIRTTF (SEQ ID NO: 1), an HLA α-chain polypeptide and a β_2 microglobulin polypeptide; the binding agent binds the HLA PHOX2$_{BQYNPIRTTF}$ complex presented by HLA-A*24:02, HLA-A*23:01, and HLA-B*14:02; and the binding agent is an antibody or antibody fragment.

2. The binding agent of claim 1, wherein the antigen-binding site binds to the HLA PHOX2B$_{QYNPIRTTF}$ complex with a dissociation constant (K_D) equal to or less than about 500 nM, equal to or less than about 200 nM, or equal to or less than about 13 nM.

3. The binding agent of claim 1 or claim 2, wherein the binding agent is not MHC-restricted.

4. The binding agent of any one of claims 1-3, wherein the binding agent comprises:
a) a V_L comprising a CDR-L1 region as set forth in SEQ ID NO: 6, a CDR-L2 region as set forth in SEQ ID NO: 7, and a CDR-L3 region as set forth in SEQ ID NO: 8; and
b) a V_H comprising a CDR-H1 region as set forth in SEQ ID NO: 9, a CDR-H2 region as set forth in SEQ ID NO: 10, and a CDR-H3 region as set forth in SEQ ID NO: 11.

5. The binding agent of claim 4, wherein the binding agent comprises a V_H and a V_L, wherein the V_H has at least 90% sequence identity to SEQ ID NO: 5, and wherein the V_L has at least 90% sequence identity to SEQ ID NO: 4.

6. The binding agent of any one of claims 1-5, wherein the binding agent is an antibody.

7. The binding agent of any one of claims 1-6, wherein the binding agent comprises a V_H and a V_L, wherein the V_H is fused to the V_L.

8. The binding agent of any one of claims 1-7, wherein the binding agent is selected from the group consisting of mAb, Fab, Fab', F(ab')$_2$, Fv, Dab single-chain antibody, scFv, CAR, ADC, KIR, BiTE, BsMAb and TFP.

9. The binding agent of claim 8, wherein the binding agent is a single chain variable fragment (scFv).

10. A chimeric antigen receptor (CAR) comprising the binding agent of any one of claims 1-9.

11. A Killer Ig-Like receptor (KIR) comprising the binding agent of any one of claims 1-9.

12. A Bispecific T cell-like Engager (BiTE) comprising the binding agent of any one of claims 1-9.

13. The CAR of claim 10, wherein the CAR comprises an intracellular signaling domain of CD3 epsilon, CD3 gamma, CD3 delta, TCR alpha, or TCR beta.

14. An isolated polynucleotide comprising a nucleic acid sequence encoding the binding agent of any one of claims 1-9, the CAR of claim 10 or claim 13, the KIR of claim 11, or the BiTE of claim 12.

15. An expression vector comprising the polynucleotide of claim 14 operatively linked to a cis-acting regulatory element.

16. A cell comprising the polynucleotide of claim 14 or the expression vector of claim 15.

17. A pharmaceutical composition comprising the binding agent of any one of claims 1-9, the CAR of claim 10 or claim 13, the KIR of claim 11, the BiTE of claim 12, the isolated polynucleotide of claim 14, the expression vector of claim 15 or the cell of claim 16.

18. A method of detecting a cancer cell, comprising contacting the cell in a sample obtained from a subject with the binding agent of any one of claims 1-9, under conditions which allow the binding agent to bind to the HLA PHOX2B$_{QYNPIRTTF}$ complex, wherein binding of the binding agent to the HLA PHOX2B$_{QYNPIRTTF}$ complex or the level thereof is indicative of the cancer cell.

19. A method of identifying cancer in a subject in need thereof, comprising:

a) detecting the presence of cancer cells in a sample obtained from the subject according to the method of claim 18;

b) identifying the subject as having cancer when cancer cells are detected.

20. The method of claim 18 or claim 19 wherein said cancer cell is a neuroblastoma.

21. Use of the binding agent of any one of claims 1-9, the CAR of claim 10 or claim 13, the KIR of claim 11, the BiTE of claim 12, the isolated polynucleotide of claim 14, the vector of claim 15, the cell of claim 16, or the pharmaceutical composition of claim 17, in the manufacture of a medicament for treating cancer.

22. The use of claim 21, wherein said cancer is neuroblastoma.

23. The use of claim 21, wherein the cancer expresses an HLA $PHOX2_{BQYNPIRTTF}$ complex presented by HLA-A*24:02, HLA-A*23:01, or HLA-B*14:02.

24. A CAR T cell comprising the CAR of claim 10 or claim 13.